Peptide mapping

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The benefit of binary pump stroke synchronization for more reliable peak identification based on improved retention time precision

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Abstract

Purpose: In this study, the goal was to show the application robustness benefits of the binary pump stroke synchronization on LC-UV peptide mapping, as an example for a specifically challenging gradient UHPLC application.

Methods: The binary pump stroke synchronization, or HPG Sync, is an optional feature of the Thermo Scientific™ Vanquish™ Binary Pump F. Reconstituted Cytochrome C digest as a proxy is analyzed repeatedly via UHPLC-UV using a Thermo Scientific™ Hypersil GOLD™ Peptide Column over 24 hours.

Results: The positive effects of the HPG Sync implementation on the retention time precision is expressed in a significant decrease in the standard deviation of the retention time of every component during the eluting gradient. The impact of the HPG Sync ON for this example of peptide mapping shows the improvements of:

- **Retention time precision**
- **Accurate peak identification**
- **Cost savings**

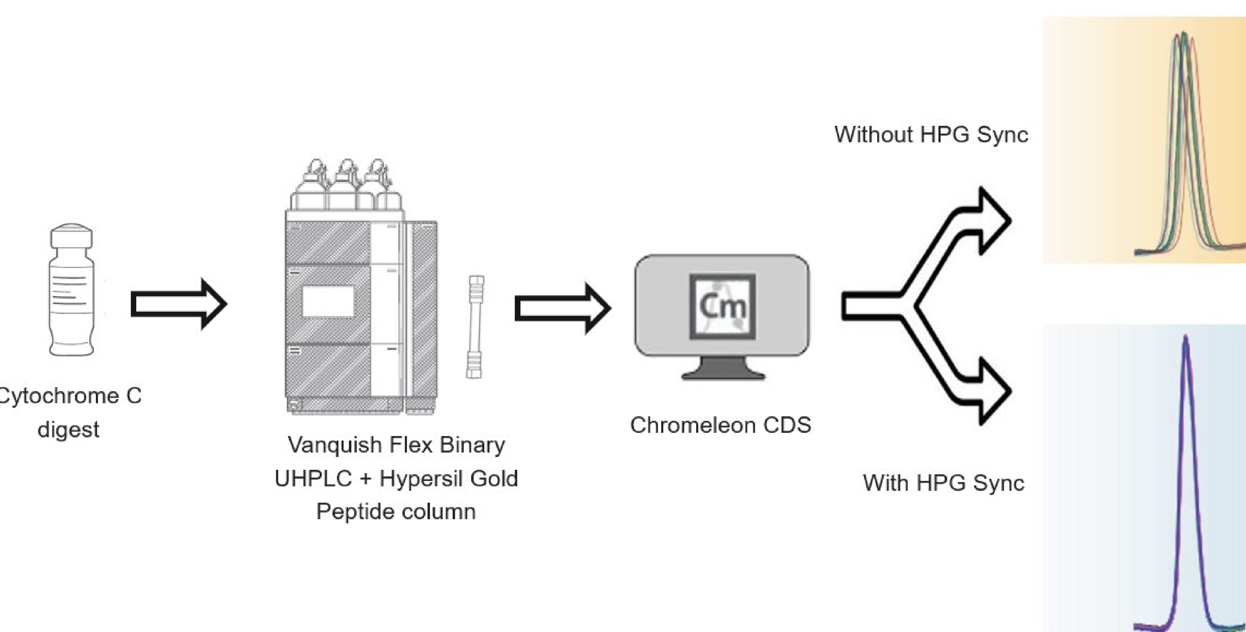

Figure 1 – The HPG Sync feature improves retention time precision.

Introduction

Retention time stability in liquid chromatography (LC) is a critical factor that significantly impacts the robustness of an analytical application. Consistent retention times ensure reproducibility, which is essential for the reliable identification and quantification of analytes. When the same compound elutes at the same time in repeated analyses, it will reduce the risk of misidentification and incorrect quantification. This reproducibility is vital for method validation, as it demonstrates that the method can produce consistent results over time, thereby ensuring the accuracy and reliability of the analytical data.

Peptide mapping is a key technique used in the characterization of proteins where peptides generated from enzymatic digestion are separated and identified. Stable retention times allow for consistent identification of peptides across different runs, which is essential for comparing results and detecting any modifications or variations in the protein structure.

The HPG Sync feature on the Thermo Scientific™ Vanquish™ Flex Binary UHPLC system is especially helpful for difficult applications like peptide separations and peptide mapping. It ensures that each injection happens at the exact same camshaft position throughout a sequence, improving consistency. As a result, retention times remain extremely stable for all components for over 24 hours. This leads to better data quality, greater confidence in results, and fewer costly errors with failed peak identification (Figure 1). Because of this reliability, a UHPLC-UV setup can often be used instead of a more expensive UHPLC-MS system, allowing peptide identification based on a previously characterized LC-MS/MS map while reducing overall costs.

Materials and methods

Sample preparation

The lyophilized sample (Cytochrome C digest) was reconstituted in 200 µL 95% water, 5% ACN with 0.1% FA. The sample was vortexed and allowed to sit for at least 10 minutes to allow reconstitution of all peptides.

Test Method

Table 1. Chromatographic conditions

Parameter	Value
Column	Hypersil GOLD Peptide, 2.1 x 150 mm, 1.9 µm (26002-152130)
Mobile Phase A	Water, 0.1% formic acid
Mobile Phase B	Acetonitrile, 0.1% formic acid
Gradient	Time [min] – B [%] 0.0 – 1 5.0 – 1 6.0 – 10 70.0 – 35 72.0 – 90 77.0 – 90 79.0 – 1 81.0 – 1 83.5 – 10 91.5 – 45 93.0 – 90 99.0 – 90 101.0 – 1 115.0 – 1
Flow rate	0.25 mL/min
Column temperature	50° C, still air 50° C active pre-heater
Injection volume	10 µL
UV detector settings	220 nm, 20 Hz data collection rate

Table 2. Used instrumentation and parts.

Module	Part Number
System Base Vanquish Horizon/ Flex	VF-S01-A-02
Vanquish Binary Pump F	VF-P10-A-01
Vanquish Split Sampler FT	VF-A10-A-02
Vanquish Column Compartment H	VH-C10-A-03
Vanquish Variable Wavelength Detector F	VF-D10-A
Semi-micro bio flow cell, 2.5 µL, 7 mm, PEEK	6077.0300
Viper TQ Pump-Autosampler Capillary (0.1x350 mm, MP35N)	6752.1035
Viper TQ active-preheater, MP35N	6732.0700
Viper TQ post-column capillary, MP35N	6732.0540

Data analysis

The Thermo Scientific™ Chromeleon™ Software 7.4 Chromatography Data System (CDS) was used for data acquisition and analysis.

Results

HPG Sync OFF versus ON

Observing the resulting chromatograms with HPG Sync OFF versus HPG Sync ON, one can immediately visualize the improvement in the trace overlays. The retention time performance already allowed for accurate peak identification, though the precision improvement can be better visualized in the zoomed regions at the beginning of the gradient (Components 1–5) for HPG Sync OFF (Figure 2A) and HPG Sync ON (Figure 2C). Additionally, the retention time precision improvement can also be seen at the end of the gradient for HPG Sync ON (Figure 2D) versus HPG Sync OFF (Figure 2B).

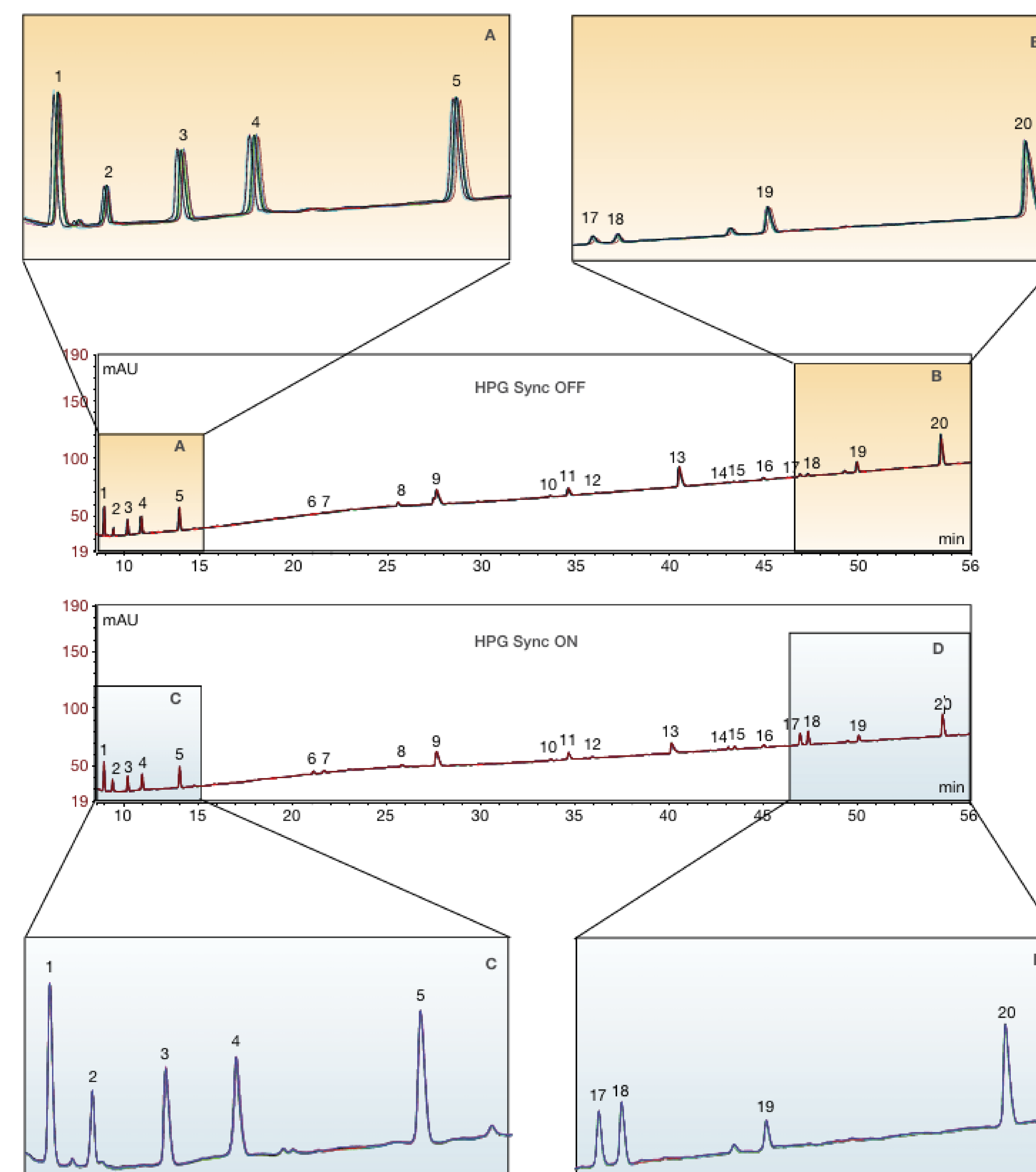

Figure 2 – Cytochrome C digest was run on the Vanquish Flex Binary UHPLC system with a Hypersil GOLD Peptide column over the course of 24 hours with n=10. The top chromatograms represent the 20 selected components throughout the gradient. Early and late sections of the chromatograms were analyzed for a zoomed visualization. (A) Early eluting components using HPG Sync OFF show slight retention time shifts as well as (B) late eluting peaks with HPG Sync OFF. In contrast, the improvement using HPG Sync ON can clearly be seen as the 10 chromatograms perfectly overlay and look like one chromatogram, not only in the above reference chromatogram but also in the zoomed (C) early eluting peaks and (D) late eluting peaks.

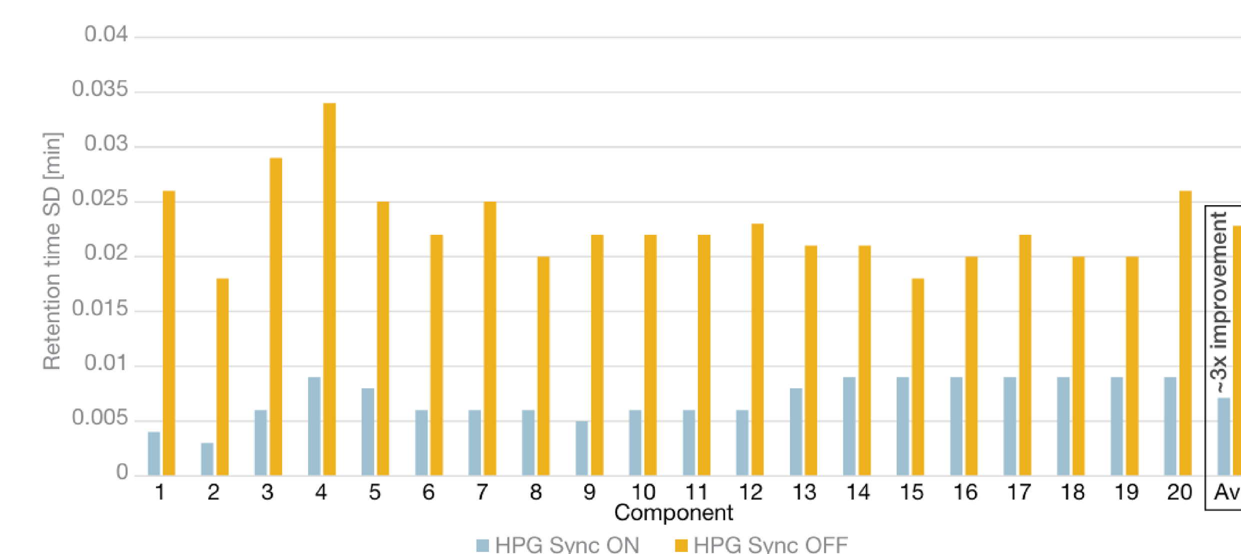

Figure 3 – Twenty components of the Cytochrome C digest were selected throughout the gradient and represented is the standard deviation for the retention time (n=10) with HPG Sync OFF versus HPG Sync ON. The average retention time standard deviation with HPG Sync OFF is ±0.023 minutes, and the average retention time standard deviation with HPG Sync ON is ±0.007 minutes for all components over the gradient, highlighting an average improvement of retention time repeatability by a factor of more than three times. With a selected user's criterion of 0.03 minutes, both modes provide passing results; the HPG Sync ON increases confidence on peak identification.

Conclusions

The positive impact of the HPG Sync ON for this example of peptide mapping was shown to improve the application robustness in terms of:

- **Retention time precision:** Retention time precision improvement can be experienced for very challenging methods requiring extremely high repeatability such as transferring this method to a QC environment.
- **Accurate peak identification:** The impact of the HPG Sync feature on the retention time repeatability makes for simpler and more accurate component identification, increasing the compatibility for automated workflow integration.
- **Cost savings:** Elimination of the MS dependency after the component characterization has taken place can substantially reduce the investment for the routine quality control workflow for peptide mapping. Retention time stability facilitates the easy transfer of component identification.

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